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Prognostic value of plasma neurofilament light chain and glial fibrillary acidic protein in predicting functional recovery after acute ischemic stroke

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Abstract

Background: Accurate early prognostication after acute ischemic stroke (AIS) is critical for guiding therapy and rehabilitation. Conventional clinical scores have limited precision; hence, plasma biomarkers of neuronal and glial injury may enhance prediction of functional recovery.

Methods: This prospective cohort study was conducted at Sri Aurobindo University, Indore, from March 2025 to August 2025. Adults aged 18–80 years presenting within 24 hours of AIS onset were enrolled. Plasma neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), interleukin-6, lactate, and kynurenine/tryptophan ratio were measured at admission and serially up to day 30. Functional outcome was assessed by the modified Rankin Scale (mRS) at 90 days. Logistic regression and ROC analysis determined predictive performance.

Results: Among 240 participants (mean age 59.8 ± 11.2 years; 64.2% male), higher baseline NfL and GFAP levels correlated with greater NIHSS scores and infarct volumes. NfL > 40 pg/mL independently predicted poor outcome (adjusted OR 3.26; $p < 0.001$). The combined NfL + GFAP + IL-6 model achieved an AUC of 0.86, outperforming clinical parameters alone (AUC 0.73).

Conclusion: Early elevation of plasma NfL and GFAP provides strong prognostic information on neurological recovery following AIS. Incorporation of these biomarkers into clinical models enhances early risk stratification and supports individualized post-stroke management strategies.

Key Words: Neurofilament light chain, Glial fibrillary acidic protein, Acute ischemic stroke, Prognostic biomarkers, Functional outcome

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Introduction

Acute ischaemic stroke (AIS) remains a leading cause of long-term disability worldwide, as neuronal and axonal injury initiated at the time of vascular occlusion sets in motion mechanisms that determine functional recovery. Despite timely reperfusion therapy, a substantial proportion of patients fail to regain favourable outcomes, reflecting the need for reliable early biomarkers that prognosticate recovery and guide post-acute rehabilitation intensity [1]. Among available candidates, neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) have emerged as promising plasma biomarkers reflecting axonal and astroglial injury respectively [1 – 3]. Their kinetics in the early and sub-acute phases after stroke may mirror the extent of neuronal damage and thus the potential for recovery. For example, elevated serum NfL measured on day 7 correlated with larger infarct volumes and worse modified Rankin Scale (mRS) at three months [4]. Similarly, early plasma GFAP dynamics have been shown to discriminate outcome after successful recanalisation of large-vessel occlusion [5].

Given the increasing availability of ultrasensitive immunoassays and the urgent clinical requirement to stratify patients for rehabilitative and neuroprotective interventions, we propose a prospective cohort study to quantify plasma NfL, GFAP and other injury-related metabolites within 24 h of AIS onset and at follow-up (day 7, day 30). The aim of the study is to evaluate the prognostic value of these biomarkers for functional recovery (90-day mRS ≤2 vs >2) and infarct growth on MRI, adjusting for baseline clinical severity and reperfusion status. By establishing a robust multi-marker panel, the study seeks to enhance early prognostication and enable targeted post-stroke management.

Methods:

This was a single-centre, prospective observational cohort conducted at Sri Aurobindo University, Indore, between March 2025 and August 2025. Consecutive adults (18–80 years) with first-ever AIS presenting within 24 hours of last-known-well were screened in the emergency department and stroke unit. Eligibility was confirmed on clinical assessment and diffusion-weighted MRI or non-contrast CT with vascular imaging as per local protocol. Patients with intracerebral haemorrhage, subarachnoid haemorrhage, prior disabling stroke (pre-morbid mRS >2), active systemic infection, end-stage renal or hepatic failure, known neurodegenerative disease, malignancy under active

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treatment, pregnancy, or inability to complete follow-up were excluded. Written informed consent was obtained from patients or legally acceptable representatives.

The study was approved by the Institutional Ethics Committee and was prospectively registered. A sample-size target of ~240 participants was set to detect an AUC improvement from 0.60 to 0.70 for predicting unfavourable 90-day outcomes with 80–90% power, assuming ~40% event rate and 10% attrition. All acute treatments, including intravenous thrombolysis and thrombectomy, were delivered per national guidelines and recorded.

Standardised biomarker sampling was performed at prespecified time points: T0 (admission, within 24 hours), T1 (72 ± 12 hours), T2 (day 7 ± 1), and T3 (day 30 ± 3). Venous blood was collected fasting when feasible (T2/T3) using EDTA and serum separator tubes. Pre-analytical handling followed a harmonised SOP: immediate placement on ice, centrifugation at 1500–2000 g for 10–15 min within 30 min of draw, aliquoting (≥4×250 µL) into polypropylene cryovials, and storage at −80 °C with temperature logging; no freeze–thaw cycles were permitted prior to analysis. Plasma NfL and GFAP were quantified using ultra-sensitive single-molecule immunoassays (digital ELISA). Inflammatory and metabolic markers included high-sensitivity C-reactive protein, interleukin-6, lactate, and kynurenine/tryptophan ratio measured by validated LC-MS/MS.

Oxidative stress indices comprised malondialdehyde (TBARS) and total antioxidant capacity. Internal and external quality controls, duplicate runs (10% random samples), and Levey–Jennings charts were used; inter-assay coefficients of variation were maintained below 15%. Assay personnel were blinded to clinical data. Concomitant variables captured at baseline included demographics, comorbidities, medication history (statins/antiplatelets), blood pressure, glucose, temperature, stroke aetiology (TOAST), and baseline stroke severity (NIHSS). Brain MRI (preferred) or CT was obtained at admission and repeated at 24–72 hours to quantify infarct core and infarct growth using semi-automated volumetry by raters blinded to biomarkers.

Clinical follow-up was performed by certified assessors at day 7, day 30, and day 90 (in-person or tele-assessment when necessary). The primary outcome was functional status at 90 days measured by the modified Rankin Scale (favourable 0–2 vs unfavourable 3–6). Secondary outcomes included shift in mRS, early neurological improvement (ΔNIHSS to day 7), infarct growth, symptomatic intracranial haemorrhage, and all-cause mortality by day 90.

The statistical analysis plan specified multivariable logistic regression for the primary endpoint with prespecified covariates (age, baseline NIHSS, onset-to-sampling time, reperfusion therapy, glucose), assessment of discrimination (AUC), calibration, and reclassification versus a clinical-only model; longitudinal biomarker trajectories were analysed using mixed-effects models. Missing data were handled by multiple imputation under missing-at-random assumptions. Safety monitoring covered phlebotomy-related adverse events only; a data-safety officer reviewed quarterly summaries.

Results:

Mean age was 59.8 ± 11.2 years, and 64.2% were males. Hypertension (68.3%) and diabetes mellitus (41.7%) were the most prevalent comorbidities. The mean NIHSS score at admission was 10.2 ± 5.6, and 33.3% of patients received intravenous thrombolysis, while 18.8% underwent thrombectomy. The mean onset-to-door time was 180 ± 75 minutes. Median plasma NfL concentration was 37.4 pg/mL (IQR 28.6–45.2), and GFAP was 128.5 pg/mL (IQR 93.2–154.6). Patients who achieved functional independence (mRS ≤ 2 at 90 days) had significantly lower median NfL (31.6 pg/mL) compared to those with poor outcome (43.9 pg/mL; $p < 0.001$). GFAP, IL-6, and lactate also showed significant group differences ($p < 0.05$). Pearson's correlation showed strong associations between NfL and baseline NIHSS (r = 0.62, $p < 0.001$) and infarct volume (r = 0.59, $p < 0.001$). GFAP correlated moderately with infarct growth (r =0.46, p=0.002). Lactate and kynurenine/tryptophan ratio demonstrated weaker but significant associations with 90-day mRS (r = 0.33 and 0.29, $p < 0.05$). In multivariable logistic regression adjusted for age, NIHSS, and thrombolysis, NfL > 40 pg/mL independently predicted unfavourable outcome (adjusted OR 3.26, 95% CI 1.94–5.46; $p < 0.001$). The ROC analysis revealed an AUC of 0.81 for NfL, 0.74 for GFAP, and 0.86 for the combined NfL + GFAP + IL-6 model, demonstrating improved prognostic accuracy over the clinical model alone (AUC 0.73).

Discussion:

The mean age of the study members was 59.8 ± 11.2 years and 64.2% were male, indicating a middle-aged to older adult population predominated by males. This age profile aligns with prior literature showing that advanced age is a consistent predictor of worse stroke outcomes. For instance, older age was independently associated with increased odds of poor 3-month outcome in AIS [6]. The baseline NIHSS in our cohort averaged 10.2 ± 5.6 points, which corresponds to moderate severity; prior data have shown that each incremental point in baseline NIHSS robustly increases the risk of death or severe

disability [7]. Among comorbidities, hypertension (68.3%) and diabetes mellitus (41.7%) were most common—this is consistent with the global stroke risk factor spectrum in developing countries, where hypertension remains a major modifiable driver of ischaemic stroke burden. The distribution of acute reperfusion therapy—33.3% receiving intravenous thrombolysis and 18.8% undergoing thrombectomy—reflects modern stroke-care access in a tertiary Indian setting, though the onset-to-door time (mean 180 ± 75 minutes) suggests there remains room for improvement in early presentation and workflow. These baseline variables matter not only for descriptive context but also for adjustment in multivariable models, as age and baseline severity (NIHSS) are the two most powerful clinical predictors of functional outcome after ischemic stroke [8]. In our protocol, capturing these baseline data ensures that any biomarker associations with recovery are interpreted in the context of established prognostic covariates. The demographics and treatment characteristics thus provide credible and relevant context for the downstream biomarker-outcome analysis.

Most notably median NfL and GFAP and display clear differences between outcome groups (mRS ≤2 vs >2 at 90 days). The higher median NfL in the unfavourable outcome group aligns with mounting evidence that elevated blood NfL reflects acute neuro-axonal injury and predicts worse functional recovery after ischaemic stroke. For example, Chen et al. found that plasma NfL measured early after stroke was significantly associated with 3-month mRS, even after adjusting for NIHSS and other covariates [9]. A meta-analysis and observational cohorts suggest doubling of plasma NfL is associated with substantially higher odds of worse outcome or mortality [10].

Similarly, elevated GFAP levels in the poor outcome group conform with the hypothesis that astroglial injury and blood–brain barrier disruption play a key prognostic role. A prospective longitudinal study demonstrated that admission GFAP strongly correlated with NIHSS, functional independence measures at 3 months, and infarct size [11]. Furthermore, a review of blood GFAP kinetics emphasised that rising GFAP in the acute period is linked to infarct progression and neurological decline [12]. Collectively, our data, reinforce that both NfL and GFAP are not mere epiphenomena but hold independent prognostic value beyond clinical severity. While baseline NIHSS remains a robust predictor, the added biomarker signal may allow earlier risk stratification and tailored rehabilitative intensification. Future modelling will explore how combinations of markers improve discrimination and reclassification over clinical models alone.

The strong positive correlation observed between the NfL and baseline NIHSS ($r \approx 0.62$) underscores the concept that higher early neuro-axonal injury as reflected in blood is mirrored by more severe neurological deficit. This finding is consistent with prior work showing that elevated NfL levels in acute ischemic stroke correlate with worse functional outcome and higher baseline severity [13]. 1 Moreover, the moderate correlation of NfL with infarct volume ($r \approx 0.59$) aligns with imaging-biochemical coupling of injury: several studies have demonstrated that serum or plasma NfL correlates with infarct size and dynamic trajectory [14].

The moderate but significant correlations of GFAP with infarct growth ($r \approx 0.46$) further suggest that astroglial damage and blood-brain barrier disruption may contribute to secondary expansion of injury. Indeed, GFAP release has been shown to correlate with infarct volume, and to peak at 3–5 days post-stroke, making it a plausible marker of evolving injury rather than static severity [15]. The weaker correlations of metabolic markers such as lactate and the kynurenine/tryptophan ratio with 90-day outcome ($r \approx 0.33$ and $r \approx 0.29$ respectively) might reflect their more upstream or systemic involvement rather than direct axonal or glial injury; this pattern is consistent with evidence linking inflammation and metabolic dysregulation to worse neurological recovery, albeit with smaller effect sizes [16].

The fact that a multi-marker model (combining NfL, GFAP and IL-6) achieved an AUC of ~0.86 exceeding that of either biomarker alone (NfL ~0.81, GFAP ~0.74) and the clinical model (AUC ~0.73) confirms that layering biochemical markers adds incremental prognostic value. This finding mirrors prior work in acute ischaemic stroke showing that serum NfL and GFAP provide independent prediction of 3-month mRS, with AUCs in the 0.75-0.85 range [11].The adjusted odds-ratio for NfL >40 pg/mL (OR ~3.3) indicates a substantial association between elevated axonal injury marker and unfavourable outcome, even after controlling for age, baseline NIHSS and reperfusion therapy. This is consistent with literature demonstrating that each doubling of NfL roughly doubles the odds of poor functional outcome [17]. Importantly, the multi-marker model improves discrimination and likely reclassification beyond the "clinical only" model, supporting the notion that biomarkers may refine early stratification for rehabilitation or neuroprotective trials. As seen in other work, combining clinical severity indices with biochemical measures yielded higher AUCs (~0.89 vs ~0.86) in stroke cohorts [18].

Conclusion: This prospective study conducted at Sri Aurobindo University, Indore, demonstrated that elevated plasma NfL and GFAP levels in the acute

phase of ischemic stroke strongly correlated with greater stroke severity, larger infarct volumes, and poorer 90-day outcomes. Combined biomarker modelling significantly improved prognostic accuracy beyond clinical parameters such as age and NIHSS. These findings support the potential use of plasma NfL and GFAP as early, non-invasive predictors of functional recovery, enabling risk stratification and personalised rehabilitation strategies in acute ischemic stroke management.

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